

CLAIMS

1. An analytical device comprising a porous material that permits liquid to migrate therein, the device comprising in the migration direction:
- 5 (i) a first zone onto which a sample suspected of containing an analyte to be assayed can be applied,
- (ii) a second zone incorporating a non-immobilised molecule capable of specifically binding to the analyte, said non-immobilised molecule is provided with a detectable label,
- 10 (iii) a third zone capable of retarding the rate of migration of the sample and the non-immobilised molecule, and
- 15 (iv) a fourth zone incorporating in at least part of the zone an immobilised state the same type of analyte as the one to be assayed or an analogue thereof being capable of specifically binding to the non-immobilised molecule.
- 20 2. A device according to claim 1 wherein the first zone and the second zone are overlapping.
3. A device according to any of the preceding claims wherein the second zone and the third zone are overlapping.
- 25 4. A device according to any of the preceding claims wherein the third zone and the fourth zone are overlapping.
5. A device according to any of the preceding claims wherein the device comprises a fifth zone into which sample not detained during migration may be adsorbed.
- 30 6. A device according to claim 5 wherein the fourth zone and the fifth zone are overlapping.
- 35 7. A device according to any of the preceding claims wherein the second zone incorporating a non-immobilised second molecule capable of binding to a compound different from the analyte to be assayed and incapable of specific binding to the analyte to be assayed, said molecule is provided with a detectable label.

8. A device according to claim 7 wherein the fourth zone incorporating in at least part of the zone an immobilised state a compound different from the analyte to be assayed and capable of binding the non-immobilised second molecule, said compound is incapable of binding specifically to the non-immobilised molecule capable of specifically binding to the
5 analyte.
9. A device according to any of the preceding claims wherein the molecule in the second zone is selected from the group consisting of antibodies and receptors.
- 10 10. A method according to any of the preceding claims wherein the first zone, the second zone, the third zone, the fourth zone and the fifth zone comprises of a porous material, said porous material is selected from the group consisting of a nitrocellulose membrane, cellulose, a polymer such as nylon, polyvinylidene fluoride or latex, glass fibre, woven
15 fibres, non-woven fibres and a chromatographic gel membrane.
11. A method according to claim 10 wherein the average pore size of the porous material is in the range of 10-10.000 nm.
12. A method according to any of claims 10-11 wherein the capacity of the porous material
20 to bind proteins is in the range of 1-400 $\mu\text{g}/\text{cm}^2$.
13. A method according to any of claims 10-12 wherein the capillary flow-rate of the porous material is in the range of 50-250 sec/4 cm.
- 25 14. A device according to any of the preceding claims wherein the analyte or analogue being immobilised to the fourth zone through a spacer molecule
15. A device according to claim 14 wherein the spacer molecule is selected from the group consisting of a peptide, a polypeptide and a protein.
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16. A device according to any of the claims 14 or 15 wherein the spacer molecule is bovine serum albumin.
17. A device according to any of the claims 15 or 16 wherein the spacer molecule and the
35 analyte or analogue being immobilised to the fourth zone is coupled using CMO and/or HMS.
18. A device according to any of the preceding claims wherein the capability of retarding the sample and the specific binding molecule of the third zone is provided by changing the

length of the porous material used in the third zone, changing the porosity of the porous material and/or adding at least one substance.

19. A device according to claim 18 wherein the sample and the specific binding molecule is
5 retarded by changing the length of the third zone relative to the length of the first, second and fourth zones.

20. A device according to any of the preceding claims wherein the third zone constitute 1-
10 99% of the porous material used in the first zone, second zone, third zone and fourth zone.

21. A device according to any of the preceding claims wherein the device further comprises a calibration zone.

15 22. A device according to claim 21 wherein the calibration zone is located downstream from the fourth zone and upstream from the fifth zone.

23. A device according to claims 20 or 21 wherein the calibration zone has immobilised thereon polyclonal or monoclonal antisera specific for the labelled non-immobilised
20 molecule capable of binding the analyte to be assayed.

24. A device according to any of the preceding claims wherein at least one zone
25 incorporating at least one ancillary compound capable of improving the flow of the liquid sample.

25. A device according to claim 24 wherein the at ancillary compound is a liquid.

30 26. A device according to any of claims 24 or 25 wherein the ancillary compound decreases non specific binding of the analyte and non specific binding of the non-immobilised specific binding molecule.

27. A device according to any of the claims 24-26 wherein the ancillary compound provides
35 a fast, consistent and quantitative release of the non-immobilised specific binding molecule.

28. A device according to any of the claims 24-26 wherein the ancillary compound provides low affinity for protein binding.

29. A device according to any of the claims 24-28 wherein the ancillary compound provides low retention of triglyceride rich samples.
- 5 30. A device according to any of the claims 24-29 wherein the ancillary compound decreases the viscosity of the sample.
31. A device according to any of the claims 27-30 wherein the ancillary compound contains chemical constituents selected from the group consisting of water, surfactant, salt, acid,
10 base, metals, sugar, proteins and lipid.
32. A device according to any of the preceding claims wherein the device comprises a solid support.
- 15 33. A device according to any of the preceding claims wherein said device is provided in the form of a dry stick.
34. An appliance carrying a multiplicity of the device according to any of claims 1-33.
- 20 35. An appliance according to claim 34 wherein an automatic, a semi-automatic and a continuous system is provided.
36. An appliance according to any of claims 34 or 35 wherein the appliance is a strip.
- 25 37. A method for assaying an analyte in a sample comprising the steps of:
- (i) applying the sample suspected of containing an analyte to a first zone,
- (ii) permitting the sample to migrate through a second zone incorporating a non-immobilised molecule capable of specifically binding to the analyte, said non-immobilised molecule is provided with a detectable label,
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- (iii) permitting the sample to migrate through a third zone capable of retarding the rate of migration of the sample and the non-immobilised molecule, and
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- (iv) permitting the sample to migrate through a fourth zone incorporating in at least part of the zone an immobilised state the same type of analyte as the one to be assayed or an analogue thereof being capable of specifically binding to the non-immobilised molecule.

38. A method according to claim 37 wherein at least one ancillary compound capable of improving the flow of the sample is added.
- 5 39. A method according to claim 37 wherein at least one ancillary compound is incorporated into at least one zone.
40. A device according to claims 38-39 wherein the ancillary compound decreases non-specific binding of the analyte and non-specific binding of the non-immobilised specific
10 binding molecule.
41. A device according to any of claims 38-40 wherein the ancillary compound provides a fast, consistent and quantitative release of the non-immobilised specific binding molecule.
- 15 42. A device according to any of claims 38-41 wherein the ancillary compound provides low affinity for protein binding.
43. A device according to any of claims 38-42 wherein the ancillary compound provides low retention of triglyceride rich samples.
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44. A device according to any of claims 38-43 wherein the ancillary compound decreases the viscosity of the sample.
45. A device according to any of claims 38-44 wherein the ancillary compound contains
25 chemical constituents selected from the group consisting of water, surfactant, salt, acid, base, metals, sugar, proteins and lipid.
46. A method according to any of claims 37-45 wherein the analyte to be assayed is a steroid selected from the group consisting of a progestagen, an estrogen and an androgen.
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47. A method according to claim 46 wherein the progestagen to be assayed is progesterone.
48. A method according to claim 47 wherein the sample to be assayed is containing 0-50
35 ng/ml of progesterone.
49. A method according to any of claims 37-48 wherein the specific binding molecule is selected from the group consisting of antibodies and receptors.

50. A method according to claim 49 wherein the antibodies are monoclonal antibodies.
51. A method according to any of claims 37-50 wherein the first zone, the second zone, the third zone, the fourth zone and the fifth zone comprises of a porous material, said
5 porous material is selected from the group consisting of a nitrocellulose membrane, cellulose, a polymer such as nylon, polyvinylidene fluoride or latex, glass fibre, woven fibres, non-woven fibres and a chromatographic gel membrane.
52. A method according to claim 51 wherein the average pore size of the porous material
10 is in the range of 10-10.000 nm.
53. A method according to any of claims 51-52 wherein the capacity of the porous material to bind proteins is in the range of 1-400 $\mu\text{g}/\text{cm}^2$.
- 15 54. A method according to any of claims 51-53 wherein the capillary flow-rate of the porous material is in the range of 50-250 sec/4 cm.
55. A method according to any of claims 37-54 wherein the detectable label is selected from the group consisting of dyes, enzymes, fluorescent compounds, chemiluminescent
20 compounds, radioactive labels and metals.
56. A method according to claim 55 wherein the detectable label is selected from the group consisting of gold, silver, carbon, fluorescent latex beads and dyed latex beads.
- 25 57. A method according to any of claims 37-56 wherein the assay time is less than 15 min.
58. A method according to any of claims 37-57 wherein the sample to be assayed is mammalian physiological fluid.
- 30 59. A method according to claim 58, wherein the mammalian physiological fluid to be tested is selected from a group consisting of milk samples, urinary samples, blood samples and saliva samples.
60. A method according to any of claims 58-59 wherein the mammal is a cow or a human.
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61. A method according to any of claims 37-60, wherein, a device as described in claims 1-32 and an appliance as described in claims 33-35 is used.

62. A method according to any of the claims 37-61 wherein the capability of retarding the sample and the specific binding molecule of the third zone is provided by changing the length of the porous material used in the third zone, changing the porosity of the porous material and/or adding at least one substance.

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63. A device according to claim 62 wherein the sample and the specific binding molecule is retarded by changing the length of the third zone relative to the length of the first, second and fourth zones.

10 64. A device according to any of the claims 37-63 wherein the third zone constitute 1-99% of the porous material used in the first zone, second zone, third zone and fourth zone.

65. A device according to any of the claims 37-64 wherein the device further comprises a calibration zone.

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66. A device according to claim 65 wherein the calibration zone is located downstream from the fourth zone and upstream from the fifth zone.

67. A device according to claims 65 or 66 wherein the calibration zone has immobilised
20 thereon polyclonal or monoclonal antisera specific for the labelled non-immobilised molecule capable of binding the analyte to be assayed.